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Short Communication

Analysis of variation in PF83, an erythrocytic merozoite vaccine candidate antigen of *Plasmodium falciparum*

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We have previously reported the identification of a 66-kDa *Plasmodium knowlesi* late-stage schizont protein (PK66) by monoclonal antibodies that inhibit in vitro multiplication of *P. knowlesi* [1]. These antibodies are effective as Fab fragments [2], and are effective against free merozoites (Thomas, unpublished observation) suggesting that PK66 has a role in the invasion of erythrocytes. PK66 is processed to 44/42-kDa components at the time of merozoite release, and these smaller fragments appear to be associated with the merozoite surface [3]. When isolated in native, but not in denatured form, PK66 induced inhibitory antibody in rabbits [4] and induced protective effects in rhesus monkeys, apparently in a synergistic response with other parasite antigens [5]. An 83-kDa precursor molecule of *Plasmodium falciparum*, that we call PF83, is synthesized by late-stage schizont infected erythrocytes, is processed to a 66-kDa component at or around the time of merozoite release, and by virtue of cross-reactivity with rabbit polyclonal anti-PK66, was identified as the *P. falciparum* analogue of PK66 (results presented at the Third International Congress on Malaria and Babesiosis, Annecy, France, 1987). The sequence of AMA-1, a *P. falciparum* merozoite antigen, has recently been reported [6] and we have shown that AMA-1 and PF83 represent the same molecule (Waters et al., manuscript submitted). PK66 is a merozoite surface antigen associated with the apical prominence [7,8] and the distribution of the *P. falciparum* analogue appears to be very similar (A. Thomas, unpublished observations).

We are interested in determining the potential of PF83 as a *P. falciparum* vaccine antigen. As a first step in this direction we have analyzed the variation in four strains of PF83 routinely cultured in our laboratory, and we compare these sequences with that of the FC27 strain that has recently been reported [6].

Genomic DNA from cloned *P. falciparum* strains CAMP (Malaysian isolate cloned at Walter Reed Army Institute of Research), 7G8 [9], Thai Tn [10] and FCR3 [11] was used as template for polymerase chain reaction (PCR) reactions primed with oligonucleotides from the extreme N and C termini of PF83. PCR reactions were digested with *Eco*RI to generate frag-

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Note Nucleotide sequence data reported in this paper have been submitted to the GenBank[TM] data base with the accession numbers M34552, M34553, M34554 and M34555

Note The views of the author do not purport to reflect the position of the Department of the Army or the Department of Defense.

ments of approximately 500 and 1500 bp for each strain, and these were cloned into the plasmid pGEM 3 (Promega) for double stranded dideoxy DNA sequencing [12]. Alignment of these DNA sequences with FC27 and the partial sequence for NF7 that was also reported [6] (not shown) revealed only limited differences between these strains. There are no deletions or additions. Nine of a total of 12 third base substitutions result in an amino acid substitution, suggesting that these substitutions are being positively selected for.

In Fig. 1, the predicted amino acid sequences for these strains are aligned. For ease of alignment all amino acids at which substitutions occur are

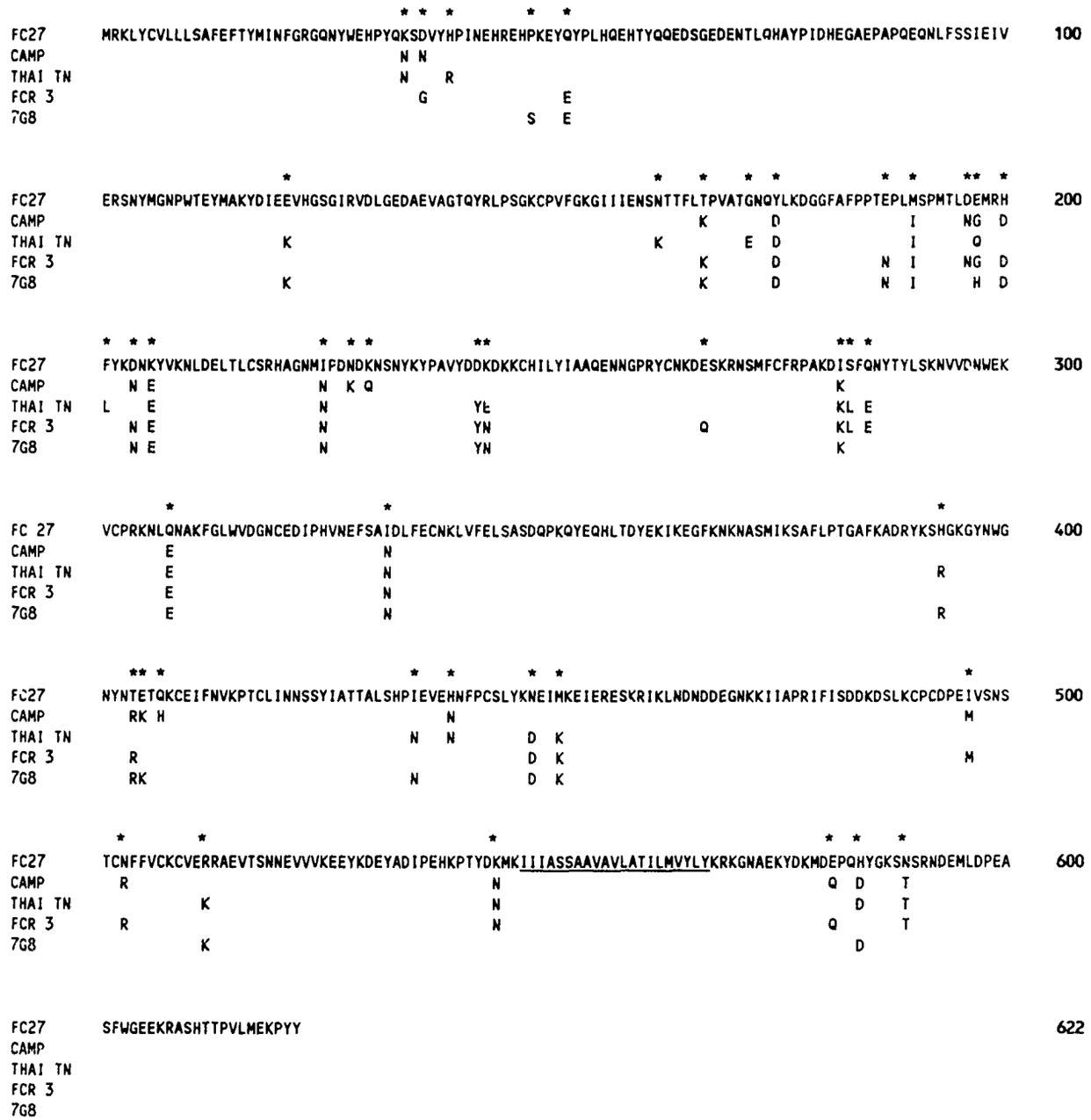

Fig 1 Alignment of predicted protein sequences from the five cloned strains of *P falciparum* for which complete PF83/AMA-1 sequences are available. Asterisks denotes residues at which a substitution occurs and the predicted trans-membrane sequence is underlined.

marked with an asterisk. Proline residues and, in particular, cysteine residues are highly conserved in the analogous molecule from other species of malaria [6,13]. It is noteworthy that none of the cysteine residues, and only a single proline residue (in an N-terminal segment that has so far proved to be unique to *P. falciparum*) are substituted in any of the strains of *P. falciparum* we compare here. These structurally important residues are likely to be critical to the correct folding, and hence conformation, of PF83. In recognition of the dependency of the protective effect of PK66 on retention of authentic conformation, we are currently attempting to express PF83 in eukaryotic systems that may reproduce the correct configuration. The predicted cytoplasmic region of PF83 may be involved in signal transduction during merozoite invasion of erythrocytes. The cytoplasmic region that lies immediately C-terminal to the membrane-spanning region contains three substitutions. These appear in more than one strain. That the extreme C-terminal region may be functionally important is suggested by the absence of substitutions within *P. falciparum*, its identity to the sequences of *P. knowlesi* and *Plasmodium fragile* and the fact that it differs from the equivalent *P. chabaudi* region by only two conservative amino acid substitutions.

Overall, variation between FC27 and each of the other four complete *P falciparum* sequences is approximately 4% at both the amino acid and DNA level. Within this pattern of variability there are seven substitutions common to CAMP, Thai Tn, FCR3 and 7G8 that are not found in the FC27 sequence. In this respect it may be noteworthy that a familial similarity has also been noted within the MSA2 allele of the geographically diverse CAMP, Thai Tn, FCR3 and 7G8 strains (Thomas et al., manuscript submitted). The distribution of the variation is not random. In particular, a relatively hot region of variation is apparent between amino acids 160 and 210, and much of the remaining variability is distributed in small clusters. The localized amino acid residue pairs 167 and 200, 242 and 243, 393 and 435, 448 and 450, 496 and 503, 544 and 589 co-vary. It is possible that some or all of these pairs are inter-dependant, in that variation in one member of the pair is always associated with variation in the other member.

We have shown that in five strains fully sequenced to date there is only limited variability of PF83. This variability may not compromise the potential of PF83 as a vaccine component, given that strain variation within the equivalent antigen of *P. knowlesi* did not appear to affect recognition by the inhibitory mAb [5,8] and that challenge of rhesus that had been immunized with PK66 did not result in the proliferation of PK66 mutants [5].

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